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Published
With international search report.

(54) Title: METHOD OF TREATING INHIBITION OF DIPEPTIDYL AMINOPEPTIDASE TYPE IV

(57) Abstract

A method of treating, in a human patient, a disease state associated with inhibition of DP-IV by a protein by interfering with the inhibition caused by the protein.

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METHOD OF TREATING INHIBITION OF DIPEPTIDYL AMINOPEPTIDASE TYPE IV

Background of the Invention

This invention relates to treating diseases associated with inhibition of physiologically significant enzymes.

Dipeptidyl aminopeptidase ("DP-IV") is a serine protease (EC number 3.4.14.5) present in many microbes, mammalian cells, and tissues, e.g., renal tubule cells, intestinal epithelium, and blood plasma. It is also present on the surfaces of human CD-4+ and some CD-8+ T-cells, and in low amounts in the central nervous system. It is thought to be involved in T-cell activation and immune regulation. Patients infected with HIV, the virus believed to be the causative agent of Acquired Immune Deficiency Syndrome (AIDS), exhibit significantly lowered DP-IV activities.

Summary of the Invention

The present invention features a method of treating, in a human patient, a disease state associated with inhibition of DP-IV by a protein by interfering with the inhibition caused by the protein.

In preferred embodiments, the disease state involves immunosuppression, e.g., such as that associated with HIV infection. Preferably, the method involves interfering with the HIV protein Tat, a protein encoded by HIV which inhibits antigen-induced, but not mitogen-induced, lymphocyte proliferation in cell culture systems. We have discovered that, in AIDS patients, Tat causes DP-IV inhibition and thus immunosuppression. Where the deleterious DP-IV inhibition by the Tat protein involves binding of DP-IV to Tat, the method of the inventi n

preferably involves interfering with that binding, e.g., by competitive inhibition using a substance capable of binding to Tat to inhibit DP-IV-Tat binding; a preferred substance includes DP-IV or a Tat-binding fragment or analog thereof.

Our discovery of the DP-IV inhibiting effect of Tat, and the consequent deleterious suppression of the immune system, also makes possible a method of improving immune function in a human patient, by administering to the patient an immune function improving amount of DP-IV. In a patient infected with HIV, such administration can serve the dual therapeutic functions of "soaking up" harmful circulating Tat protein, while at the same time replenishing depleted, immuno-stimulating DP-IV.

Our discovery of the Tat-DP-IV interaction also permits the exploitation of that interaction in the treatment of a different class of diseases, in which immunosuppression is desired, e.g., autoimmune diseases such as rheumatoid arthritis and SLE, as well as malignancies such as T-cell leukemias. That method of effecting immunosuppression in a human patient in need of immunosuppression includes administering to the patient an immunosuppressive amount of Tat protein or a DP-IV-binding fragment or analog thereof.

The invention also provides an assay for measuring the amount of Tat protein, and thus HIV activity, in a sample, e.g., a blood sample from an AIDS patient being monitored, that includes the steps of adding a pre-determined amount of DP-IV to the sample and measuring the level of DP-IV activity as an inverse measure of the amount of Tat protein in the sample. Preferably, the level of DP-IV activity is measured colorimetrically.

The invention provides an effective treatment for patients suffering from immunosuppressive diseases such as AIDS in which DP-IV activity is inhibited. The course of the therapy can be monitored readily by measuring the amount of Tat protein in a serum sample taken from the patient to which DP-IV has been added; the extent to which DP-IV activity is inhibited is a measure of the amount of Tat protein in the sample. The invention also provides an effective means of inducing immunosuppression in patients suffering from certain diseases by administering Tat protein.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of the Preferred Embodiments

We first briefly describe the drawings.

Drawings

Fig. 1 is the nucleotide sequence and deduced amino acid sequence of DP-IV.

Fig. 2 is the amino acid sequence of Tat protein.

The Tat-DP-IV Interaction

We have discovered that Tat protein found in patients infected with AIDS inhibits the activity of DP-IV. As a result, when T-cells die they are not replenished at a sufficiently high rate, causing the patient to become immuno-compromised. We thus believe that HIV may act to cause T-cell depletion at least in part indirectly, by production of the Tat protein, which binds to and inhibits DP-IV, and prevents DP-IV from fulfilling its normal function in the T-cell proliferation process.

Therapy

Based on our discovery, we have devised a method for treating patients suffering from

immunosuppressive diseases such as AIDS that involves interfering with the ability of Tat protein to bind to DP-IV. One way of accomplishing this is to administer DP-IV (or a Tat-binding fragment or analog thereof) to the patient. Administration is preferably by intravenous injection, so that DP-IV is placed directly into the bloodstream. Other forms of administration (e.g., oral, topical, intramuscular, intraperitoneal, parenteral, nasal, or suppository) may also be used.

Known techniques may be used to improve the efficacy and decrease the side effects of IV-administered DP-IV. To prevent rapid removal of the enzyme from the blood by the liver, DP-IV can be modified by attachment to the enzyme of numerous polyethylene glycol (PEG) molecules. PEG modification of the enzyme could increase half-life and in addition prevent administered enzyme from triggering an unwanted immune response. PEG treatment has been employed successfully with the enzyme adenosine deaminase (produced by Enzon, Inc., New Jersey). Tat could also be removed by administration of antibody (monoclonal or polyclonal) to Tat. Specificity can be enhanced by producing the antibody using, as an immunogen, a region of Tat which binds specifically to DP-IV. It has been shown that transition state analogs of DP-IV substrates bind tightly to and inhibit DP-IV; these analogs, described in Bachovchin et al. U.S. Serial No. 510,274, filed April 17, 1990, hereby incorporated by reference, contain the DP-IV-binding unit Ala-boro Pro. Antibodies to these transition state analogs can be expected to bind specifically to Tat.

The amount of DP-IV administered is selected to cause the total circulating DP-IV level to be higher than the Tat protein level. In this way, a portion of the DP-IV is available to bind to the Tat protein,

thereby causing it to be eliminated from the body, and the remaining portion is available to replenish depleted DP-IV levels in the body. Once normal DP-IV levels are restored, the body can begin replenishing depleted T-cells. Once normal immune function has been restored, the immune system may be able to more effectively combat HIV.

The proper DP-IV dosage is selected by first measuring the level of Tat protein in the patient. This is preferably done by titration, i.e., by adding a pre-determined amount of DP-IV to a serum sample taken from the patient and then measuring the extent to which the Tat protein inhibits DP-IV activity, using standard protocols. Once the Tat protein level is known, an excess of DP-IV (e.g., 2-3 times the molar Tat level) is administered to the patient, in a conventional pharmaceutically acceptable carrier, e.g., saline. Typical dosages are 1 - 500 mg/kg/day. AIDS patients may require periodic, e.g., daily, administration of DP-IV for life, much as a diabetic requires regular insulin injections for life. DP-IV can be administered in conjunction with other therapies, e.g., anti-viral agents such as AZT. DP-IV administeration could also be carried in conjunction with administeration of one or more products of DP-IV enzymatic action, e.g., cleaved cytokines, to replenish those products depleted by DP-IV deficiency. Cytokines, e.g., IL-1_B and IL-2, which might be acted upon by DP-IV could be administered as well.

Both DP-IV and Tat protein have been cloned and expressed, and can be made in quantity using conventional recombinant cell growth techniques. DP-IV is described in Hong et al., Proc. Natl. Acad. Sci. USA 84:7962-66 (1987), and the nucleotide and amino acid sequence of DP-IV is shown in Fig. 1. The Tat protein

is described in Frankel et. al., Proc. Natl. Acad. Sci. USA 86:7397-7401, and the amino acid sequence of Tat protein is shown in Fig. 2. Appropriate DP-IV or Tat-binding fragments or analogs of each protein can be determined using standard screening techniques.

A second approach to therapeutically interfering with binding between Tat protein and DP-IV is to prepare a chromatography column containing DP-IV (or a Tat-binding fragment or analog thereof). Blood from the patient is then passed through the column as in kidney dialysis. The DP-IV in the column binds Tat protein in the blood, thereby removing it from the blood. The cleansed blood is then returned to the patient.

Other embodiments are within the following claims. For example, the above-described procedures can be modified to treat patients suffering from diseases characterized by an excess of white blood cells such as leukemia by substituting Tat protein for DP-IV in the therapeutic method.

CLAIMS

1. A method of treating, in a human patient, a disease state associated with inhibition of DP-IV by a protein, said method comprising interfering with said DP-IV inhibition by said protein.

2. The method of claim 1 wherein said disease state involves immunosuppression.

3. The method of claim 2 wherein said immunosuppression is associated with HIV infection.

4. The method of claim 3 wherein said protein is HIV Tat protein.

5. The method of claim 4 wherein said DP-IV inhibition by said Tat protein involves DP-IV-Tat binding, and said interfering with inhibition is carried out by interfering with said binding.

6. The method of claim 5 wherein said interfering with said binding is carried out by competitive inhibition using a substance capable of binding to Tat to inhibit DP-IV-Tat binding.

7. The method of claim 6 wherein said substance comprises DP-IV or a Tat-binding fragment or analog thereof.

8. A method of improving immune function in a human patient comprising administering to said patient an immune function improving amount of DP-IV.

9. A method of effecting immunosuppression in a human patient in need of immunosuppression, said method comprising administering to said patient an immunosuppressive amount of Tat protein or a DP-IV-binding fragment or analog thereof.

10. An assay for meausuring the amount of Tat protein in a sample comprising the steps of

adding a pre-determined amount of DP-IV to said sample; and

measuring the level of DP-IV activity as an indication of the amount of Tat protein in said sample.

11. The assay of claim 10 wherein said level of DP-IV activity is measured colorimetrically.

12. A method of removing Tat protein from a sample of human blood, comprising contacting said sample with a substance which is capable of specifically binding Tat and which is coupled to a solid support.

13. The method of claim 12 wherein said subtance is DP-IV, coupled to a column.

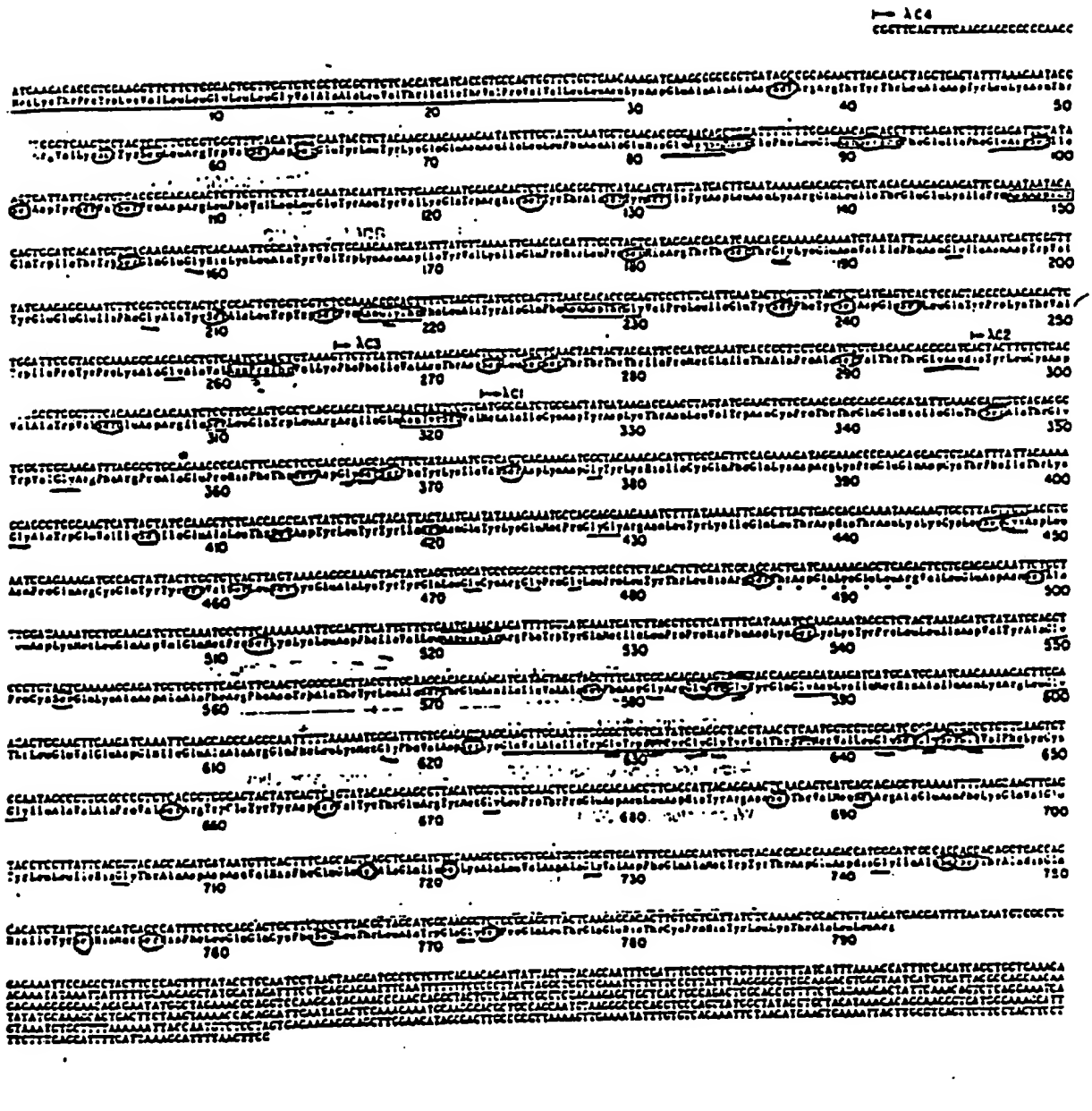

Fig. 1

1 5 10 15
Met Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser Gln Pro Lys Thr

21 38
Ala Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Phe His Cys Gln Val Cys Phe Ile Thr

47 58
Lys Ala Leu Gly Ile Ser Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Pro Pro Gln

72
Gly Ser Gln Thr His Gln Val Ser Leu Ser Lys Gln Pro Thr Ser Gln Ser Arg Gly Asp

86
Pro Thr Gly Pro Lys Glu

Fig. 2

INTERNATIONAL SEARCH REPORT

International Application No PCT/US91/03571

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

U.S. Cl.: 424/94.63 IPC(5): A61K 37/54

II FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
USA	424/94.63

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched 8

CAS, BIOSIS, APS

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No 13
A	EXPERIMENTAL CELL RESEARCH, Volume 178, issued September 1988, C. Hanski et al., "Direct Evidence for the Binding of Rat Liver DPP IV to Collagen in Vitro", pages 64-72, see entire document.	1-7
Y	CHEMICAL ABSTRACTS, Volume 112, No. 1, issued 29 January 1990, T. Aoyagi et al. "Suppression of the Activities of T-Lymphocyte-Related Enzymes in Spleen by Administration of an Immunosuppressant, 15-Deoxy-spergualin", see page 27, column 1, abstract no. 30376a, Biochem. Int. (1989), 19(4), 821-826.	1-7
Y	SCAND. J. IMMUNOL., Volume 31(4), issued April 1990, A.J. Ulmer et al., "CD26 Antigen is a Surface Dipeptidyl Peptidase IV (DPPIV) as Characterized by Monoclonal Antibodies Clone TII-19-4-7 and 4EL1C7", pages 429-435, see entire abstract, introduction and discussion.	1-7

* Special categories of cited documents: 10

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
08 August 1991	12 SEP 1991
International Searching Authority: ISA/US	Signature of Authorized Officer: Jon P. Weber

Form PCT/ISA/210 (second sheet) (rev 11-87)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y, P	BIOL. CHEM. HOPPE-SEYLER, Volume 371(8), issued August 1990, E. Schön et al., "Dipeptidyl Peptidase IV in the Immune Systems", pages 699-705, see summary, and discussion especially page 704 first paragraph.	1-7
Y, P	JOURNAL OF LEUKOCYTE BIOLOGY, Volume 48(4), issued October 1990, R.W. Barton et al., "Binding of the T Cell Activation Monoclonal Antibody Ta1 to Dipeptidyl Peptidase IV", pages 291-296, see abstract, introduction and discussion.	1-7
Y, P	PROC. NAT'L ACAD. SCI., USA, Volume 88, issued 15 February 1991, G.R. Flentke et al., "Inhibition of Dipeptidyl Aminopeptidase IV (DP-IV) by Xaa-boro-Pro Dipeptides and Use of These Inhibitors to Examine the Role of DP-IV in T-Cell Function", pages 1556-1559, see abstract, introduction and discussion.	1-7

FURTHER INFORMATI N CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [2] not required to be searched by this Authority, namely:

2. ☐ Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [3], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [4]

This International Searching Authority found multiple inventions in this international application as follows:

see attached sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

1-7

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Group I, Claims 1-7, drawn to method of treat disease state of immunosuppression by binding TAT with DP-IV, classified in Class 424, subclass 94.63.

Group II, Claim 8, drawn to method of improving immune function by administering DP-IV, classified in Class 424, subclass 94.63.

Group III, Claim 9, drawn to effecting immunosuppression by administering TAT, classified in Class 514, subclass 2.

Group IV, Claims 10-11, drawn to assay for TAT in sample by binding to DP-IV and measuring DP-IV activity, classified in Class 435, subclass 24.

Group V, Claims 12-13, drawn to method of removing TAT from blood using a DP-IV affinity column, classified in Class 435, subclass 2.